Measurement of chloramphenicol by capillary zone electrophoresis following end-column amperometric detection at a carbon fiber micro-disk array electrode

Wenrui Jin*, Xiaoying Ye, Daiqing Yu, Qian Dong

Laboratory of Analytical Science, School of Chemistry, Shandong University, Jinan 250100, China

Abstract

Capillary zone electrophoresis was employed for the measurement of chloramphenicol using end-column amperometric detection with a carbon fiber micro-disk array electrode, at a constant potential of -1.00 V vs. saturated calomel electrode. The effect of oxygen in the buffer has been investigated. It is found that when the area of the carbon fiber electrode is smaller than 1.1 mm^2, the interference of oxygen can be overcome. In this procedure deoxygenation is not necessary. The effect of pH, the concentration of the buffer and the high separation voltage across the capillary on the migration time, electrophoretic peak current and separation efficiency has been studied. The optimum conditions of separation and detection are 8.4×10^{-4} mol/l HOAc–3.2×10^{-3} mol/l NaOAc for the buffer solution, 20 kV for the separation voltage, 5 kV and 5 s for the injection voltage and the injection time, respectively. The calibration plot was found to be linear in the range 5×10^{-6} to 1×10^{-3} mol/l and the limit of detection is 9.1×10^{-7} mol/l or 1.4 fmol ($S/N=2$). The relative standard deviation is 1.1% for the migration time and 2.3% for the electrophoretic peak current. The method was applied to the determination of chloramphenicol in human serum. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: Chloramphenicol; Capillary electrophoresis; Electrochemical detection

1. Introduction

In recent years capillary zone electrophoresis (CZE) has become a powerful instrumental technique for the rapid separation and detection of a variety of complex mixtures [1–3]. The primary advantage of CZE is its ability to provide extremely high separation efficiencies in short times and to do so with relatively little sample and simple instrumentation. Amperometric detection in CZE has been demonstrated to be a highly sensitive method for the determination of a wide range of electroactive compounds [4]. In our laboratory this technique has been applied to cysteine [5], glutathione [6], purine bases [7–9], bovine serum albumin [10] and cytochrome c [11]. The theory concerning the current for the end-column amperometric detector in CZE has also been investigated [12,13]. Chloramphenicol, a broad-spectrum antibiotic, is still widely used in the treatment of a number of serious infections and is particularly effective in the treatment of central infections when these occur in neonates. High-performance liquid chromatography [14–16] and gas chromatography

*Corresponding author. Fax: +86-0531-890-2167.
E-mail address: wenrujin@jn-public.sd.cninfo.cn (W. Jin)

[17,18] have been developed for the determination of chloramphenicol.

Numerous electroactive compounds of pharmaceutical significance can, in principle, be determined by CZE following amperometric detection. Chloramphenicol has a nitro group which is electrochemically active. This characteristic has been used for polarographic determination of chloramphenicol [19]. Usually, CZE with electrochemical detection is used for those compounds oxidized at the working electrodes. In this case the interference of oxygen in the solution can not be considered. However, when the compounds, which can be reduced, are determined by CZE following the electrochemical detection, the buffer has to be deaerated before and during each run [20], because oxygen can be reduced at the same working electrode.

In this work we will present the results of end-column amperometric detection of chloramphenicol in HOAc–NaOAc by CZE using a carbon fiber micro-disk array electrode without deoxygenation. The separation was performed in a 25 μm I.D. fused-silica capillary. Using potentiostatic control of the electrode potential by means of a three-electrode system carried out the detection. The method has been used to determine chloramphenicol in human serum.

2. Experimental

2.1. Apparatus

2.1.1. Linear sweep voltammetry

A commercial polarograph (Model 83-2.5, Ningde Analytical Instruments, China) coupled with an X-Y recorder (Model 3086-11, Yokogawa Hokuskin, Japan) was used. This was connected to a cell using potentiostatic control of the electrode potential by means of a three-electrode system, which consisted of a carbon fiber array electrode as the working electrode, a Pt wire as the auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode. The reference electrode was connected to the analyte via a salt bridge filled with the same supporting electrolyte as in the cell.

2.1.2. Capillary zone electrophoresis

A reversible high-voltage power supply (Model GDY, Shandong Institute of Chemical Engineering and School of Chemistry, Shandong University, China) provided a variable voltage of 0–30 kV across the capillary with the outlet of the capillary at ground potential. Fused-silica capillaries (360 μm O.D., 25 μm I.D.) were purchased from Yongnian Optical Conductive Fiber Plant, China. They were cut to a length of 40 cm and placed between two buffer reservoirs. A high voltage was applied at the injection end, while the reservoir containing the electrochemical detection cell was held at ground potential. Separations were carried out at an applied voltage of 20 kV.

The electrochemical detection at a constant potential was performed using the end-column amperometric approach with a voltammetric analyzer (Model JF-01, Shandong Institute of Chemical Engineering and school of Chemistry, Shandong University, China). The detection cell and detector were housed in a faradaic cage in order to minimize interference from external noise sources. Electrochemical detection was carried out with a three-electrode system. The system consisted of a carbon fiber micro-disk array electrode as the working electrode, a coiled Pt wire as the auxiliary electrode, which also served as the ground for the high potential drop mentioned above across the capillary and a SCE as the reference electrode. The arrangement of the electrochemical detection cell was illustrated in detail in reference [10].

2.1.3. Carbon fiber electrodes

For linear sweep voltammetry the carbon fiber array electrode was used. A small amount of mercury was drawn into the glass capillary (ca. 0.5 mm I.D., 1 mm O.D. and 5 cm length). About 60 carbon fibers of 6 μm diameter soaked in acetone were carefully inserted into the glass capillary at the other end. The carbon fiber array was connected to a copper wire (0.4 mm diameter, 12 cm length) via the mercury junction by pushing in a copper wire. After drying, the other end of the copper wire and the carbon fiber array were bonded to the glass capillary using a low viscosity ethyl α-cyanoacrylate adhesive. The carbon fibers and the adhesive were lightly

touched with a glass bar. A glass tube (1.5 mm I.D., 8 mm O.D., 6 cm length) was put around the glass capillary, in order to protect it. The copper wire was bonded to the glass tube using epoxy. The carbon fiber array was bonded at the other end of the glass tube and protruded approximately 1 cm from the end. Then the carbon fibers were cut to 4 mm length. for capillary zone electrophoresis the carbon fiber micro-disk array electrodes were constructed using 6 μm carbon fibers. The manufacturing process is similar to the carbon fiber array electrode described above. Approximately 40 carbon fibers soaked with acetone were inserted into a fused-silica capillary (ca. 250 μm I.D., 375 μm O.D., 1.5 cm length). Next the carbon fiber array was immersed into the ethyl α-cyanoacrylate adhesive and the adhesive allowed to pass through the whole carbon fiber array into the fused-silica capillary. Then, the fused-silica capillary with the carbon fiber array was inserted into a glass capillary (ca. 0.5 mm I.D., 1 mm O.D. and 2.5 cm length) and was bonded together. Finally, the carbon fiber array protruding from the fused-silica capillary was trimmed.

Before use all carbon fiber micro-disk array electrodes were cleaned in alcohol and washed with double distilled water for 5 min by a supersonic wave cleaner. During electrophoresis, the electrodes can be directly washed with alcohol and water in the detection cell.

2.2. Reagents and solutions

A 1.00×10^{-2} mol/l stock solution of chloramphenicol was prepared by dissolving an appropriate amount of chloramphenicol in water and stored at 4°C in a refrigerator. Dilute solutions were obtained by serial dilution of the stock solution with water. All reagents were of analytical grade. All solutions were prepared with double distilled water.

2.3. Procedure

For linear sweep voltammetry the carbon fiber array electrode was directly inserted in the experimental solution containing chloramphenicol, and a linear sweep voltammogram was recorded.

In CZE, the carbon fiber micro-disk array elec-

trode was cemented onto a microscope slide, which was placed over a home-made XYZ micro-manipulator and glued in place. The position of the carbon fiber micro-disk array electrode was adjusted (under a microscope) against the end of the capillary, so that the electrode and the capillary were in contact. This arrangement allowed easy removal and realignment of both the capillary and the electrode. The other end of the capillary was inserted into a plastic syringe tip (the metal needle was previously removed) and glued in place with a small amount of epoxy glue. Before each run, the capillaries were flushed with double distilled water, 0.1 mol/l NaOH, double distilled water and the corresponding separation electrolyte by means of a syringe. In addition, the electrolyte solution at the electrochemical cell was also replaced before each run. During the experiments the separation voltage was applied across the capillary and the detection potential was applied at the working electrode. After the electro-osmotic current reached a constant value (after 20 min), the electromigration injection was carried out at 5 kV for 5 s and the electropherogram was recorded. For a capillary of 25 μm I.D., an injection volume of 1.55 nl can be calculated. The separation electrolyte in the capillary was replaced after 5 or 6 runs.

All potentials were measured vs. SCE.

3. Results and discussion

3.1. Voltammetric characteristics of chloramphenicol at the carbon fiber electrode

Usually, when a negative potential vs. SCE is applied at the working electrode, oxygen in the solution is reduced, which produces the detection noise. The linear sweep voltammograms of the buffer and chloramphenicol at a carbon fiber array electrode of 6.8 mm^2 are illustrated in Fig. 1 with and without deoxygenation. From Fig. 1 curve 1, it can be observed that there is a reduction peak of oxygen, which overlaps the reduction peak of chloramphenicol in HOAc–NaOAc of pH 5.2 (curve 2). The potential of mixture peak is at ca. −0.9 V. If the solution is deoxygenated, the oxygen peak dis-
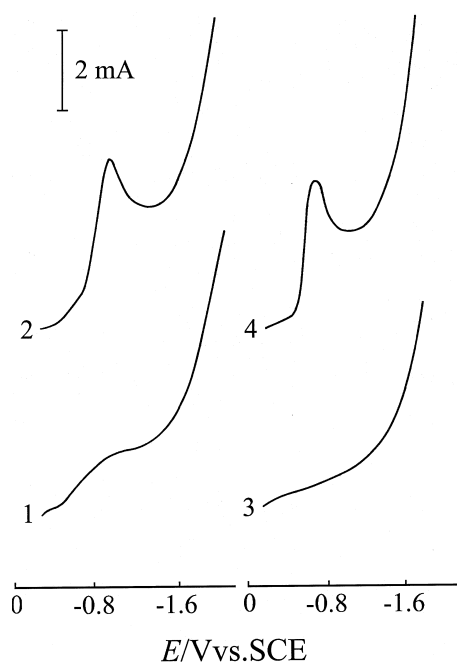
Fig. 1. Typical linear sweep voltammograms of chloramphenicol at the carbon fiber array electrode of 6.8 mm^2. 1 and 3, 8.4×10^{-3} mol/1 HOAc–3.2×10^{-2} mol/1 NaOAc at pH 5.2; 2 and 4, $(1) + 1.00 \times 10^{-3}$ mol/1 chloramphenicol; 1 and 2, without deoxygenation; 3 and 4, with deoxygenation, $v = 100$ mV/s.

appears (curve 3) and the reduction peak of chloramphenicol appears at ca. -0.6 V (curve 4). The peak current of chloramphenicol (3.1 mA) shown in curve

4 is lower than that of the mixture peak (3.8 mA) shown in curve 2. This means that oxygen in the solution can interfere with measuring the reduction signal of chloramphenicol. When smaller carbon fiber array electrodes are used, the interference of oxygen weakens. The difference of the peak potential and the peak current of chloramphenicol in both solutions with and without deoxygenation can not be observed for the electrode areas smaller than 1.1 mm^2. When a carbon fiber micro-disk array electrode of 1.2×10^{-3} mm^2 is used, the peak potential (-0.6 V) and the peak current (87.5 μA) are the same for the two cases with and without deoxygenation. This phenomenon makes the electrochemical detection of substances reduced at the working electrode simple.

3.2. Optimum conditions of CZE with end-column amperometric detection

In HOAc–NaOAc at pH 5.2 there is one reduction peak of chloramphenicol in the linear sweep voltammogram shown in Fig. 1. Therefore, the electrophoretic behavior of chloramphenicol in seven solutions of near pH 5.2 was investigated. The peak current, i_p, the migration time, t_m, the width at the half-peak, $W_{1/2}$, on the electropherograms and the number of theoretical plates, N, at different pH values are listed in Table 1. N was calculated according to the following equation:

Table 1
The values of t_m, i_p, $W_{1/2}$ and N in HOAc–NaOAc at different pH (2.00×10^{-4} mol/1 chloramphenicol, capillary: 40 cm length, 25 μm I.D.; injection, 5 kV for 5 s; separation voltage, 20 kV; detection potential, -1.00 V)

Buffer	pH	t_m (s)	i_p (nA)	$W_{1/2}$ (s)	$10^{-4}N$
2.0×10^{-3} mol/1 HOAc -2.0×10^{-3} mol/1 NaOAc	4.6	159	3.75	2.0	3.5
1.6×10^{-3} mol/1 HOAc -2.4×10^{-3} mol/1 NaOAc	4.8	159	7.50	2.0	3.5
1.2×10^{-3} mol/1 HOAc -2.8×10^{-3} mol/1 NaOAc	5.0	158	20.0	2.0	3.5
8.4×10^{-4} mol/1 HOAc -3.2×10^{-3} mol/1 NaOAc	5.2	161	21.9	2.0	3.6
5.6×10^{-4} mol/1 HOAc -3.4×10^{-3} mol/1 NaOAc	5.4	141	16.2	2.0	2.8
3.6×10^{-4} mol/1 HOAc -3.6×10^{-5} mol/1 NaOAc	5.6	127	12.5	2.0	2.2
2.4×10^{-4} mol/1 HOAc -3.8×10^{-5} mol/1 NaOAc	5.8	119	12.5	2.5	1.3

$$N = 5.54 \times \left(\frac{t_m}{W_{1/2}} \right)^2 \tag{1}$$

t_m and N are a constant between 4.6 and 5.0. There is a maximum for t_m and N at pH$=5.2$. Then they decrease with increasing pH. i_p increases and then decreases with increasing pH. Therefore, a pH of 5.2 was selected. The effect of the concentration of the buffer, C_B, on t_m, i_p,$W_{1/2}$, and N in HOAc–NaOAc is listed in Table 2. In Table 2, C_B indicates the value of the concentration of HOAc; the ratio of the concentration of HOAc to the concentration of NaOAc is 1:3.8. t_m, i_p, and N increase with increasing C_B, since the migration velocity of the substance depends mainly on the electroosmotic velocity, v_{eo}, of buffer, which is proportional to the ζ potential [21]. With increasing buffer concentration, the thickness of the electrical double layer is reduced and the ζ potential becomes smaller. Therefore, v_{eo} decreases and t_m increases. Due to "stacking effect", N increases with increasing C_B. In addition, when v_{eo} decreases, chloramphenicol has longer time to be in contact with the surface of the working electrode, which allows more chloramphenicol molecules to be oxidized on the surface of the electrode. Therefore, i_p increases with increasing C_B. We have observed that the noise increases with increasing C_B. In our experiments 8.4×10^{-4} mol/l HOAc–3.2×10^{-3} mol/l NaOAc was used because of higher i_p, larger N and lower noise.

The separation voltage, V_s, exerts an influence on t_m and N according to following relationship [22].

$$t_m = \frac{L^2}{(\mu_{ep} + \mu_{eo})V_s} \tag{2}$$

$$N = \frac{(\mu_{ep} + \mu_{eo})V_s}{2D} \tag{3}$$

Table 2
The values of t_m, i_p, $W_{1/2}$ and N at different concentrations of C_B (other conditions as in Table 1)

$10^4 C_B$ (mol/l)	t_m (s)	i_p (nA)	$W_{1/2}$ (s)	$10^{-4} N$
2.1	140	4.82	2.0	2.7
4.2	150	16.4	2.0	3.1
8.4	161	21.9	2.0	3.6
16.8	167	25.8	2.0	3.9
21.0	178	32.1	2.0	4.4

where L is the length of the separation capillary, and μ_{ep} and μ_{eo} are electrophoretic mobility and electroosmotic mobility, respectively. D is the diffusion coefficient of the solute.

Fig. 2 shows the dependence of $1/t_m$, i_p, $W_{1/2}$ and N on V_s. $1/t_m$ and N are proportional to V_s, which agrees with the theoretical Eqs. (1) and (2). With increasing V_s, $W_{1/2}$ decreases and i_p increases. Since V_s becomes higher, the migration velocity increases and the axial diffusion of the analyte becomes lower, which causes zone narrowing. In this case, the molecules of the analyte can concentrate at the working electrode. Therefore, i_p becomes larger. Nevertheless, the noise increases with increasing V_s. Therefore, 20 kV for V_s was chosen because of larger i_p and N and lower noise.

Fig. 3 shows the relationship between the detected peak current, i_p, and the applied potential, E_d. When E_d is between -0.30 and -1.00 V, i_p increases with increasing E_d. When $E_d < -1.00$ V, i_p decreases. Therefore, E_d of -1.00 V is used for the detection because of lower base line noise and fine shape of the electropherograms.

Fig. 4 shows the typical electropherograms of 2.00×10^{-4} mol/l and 5.00×10^{-6} mol/l chloram-
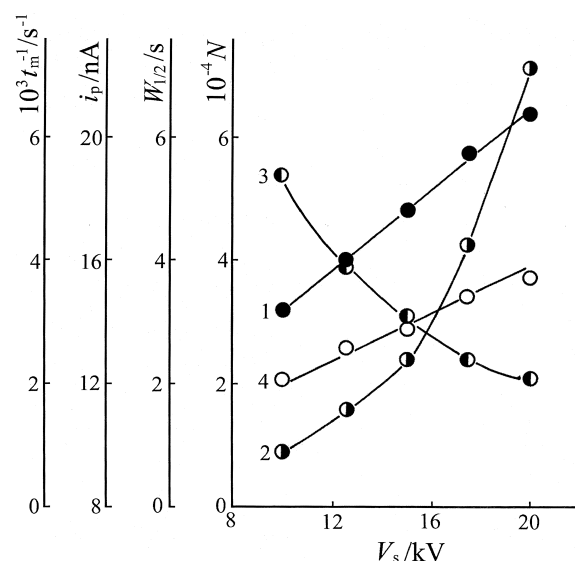

Fig. 2. Dependence of the reciprocal migration time (1), the peak current detected (2), the width at half-peak (3) and the number of theoretical plates (4) on the separation voltage. 8.4×10^{-4} mol/l HOAc-3.2×10^{-3} mol/l NaOAc. Other conditions as in Table 1.
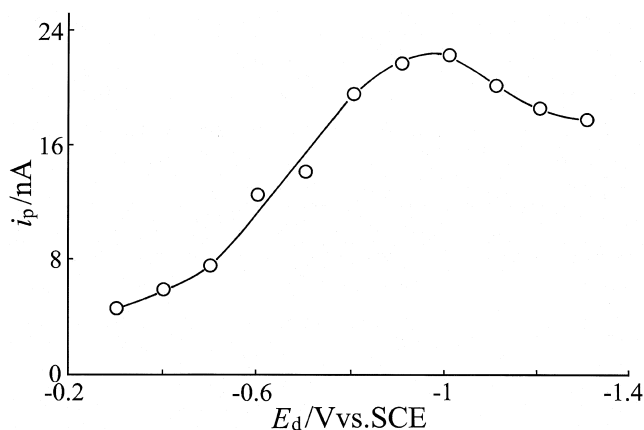
Fig. 3. Relationship between detected peak current and detection potential. Conditions as in Fig. 2.
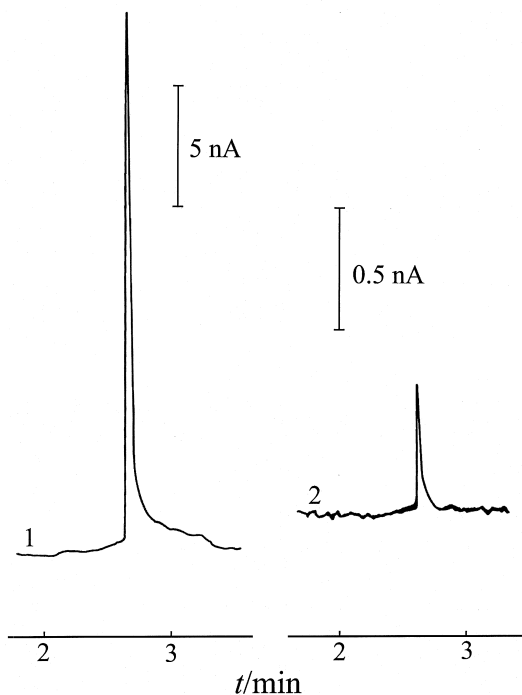

Fig. 4. Typical electropherograms of chloramphenicol. Concentration of chloramphenicol: (1) 2.00×10^{-4} mol/l; (2) 5.00×10^{-6} mol/l. Conditions as in Fig. 2.

phenicol at the optimum conditions. Small peak width and little tailing of the peak were obtained.

3.3. Reproducibility, limit of detection and linear range

The response for a series of eight injections of 2.00×10^{-4} mol/l chloramphenicol resulted in a relative standard deviation of 1.1% for t_m and 2.3% for i_p, respectively. The limit of detection is 9.1×10^{-7} mol/l (at a signal-to-noise ratio of 2), which was estimated from the electropherograms obtained for 5.00×10^{-6} mol/l chloramphenicol (see Fig. 4, curve 2), or 1.4 fmol for the injected volume calculated.

A linear relationship holds between the peak current detected and concentration in the range of 5.00×10^{-6} to 1.00×10^{-3} mol/l. Least-squares treatment of these data yielded a slope of 115 pA μmol^{-1}l and a correlation coefficient of 0.9996.

3.4. Determination of chloramphenicol in human serum
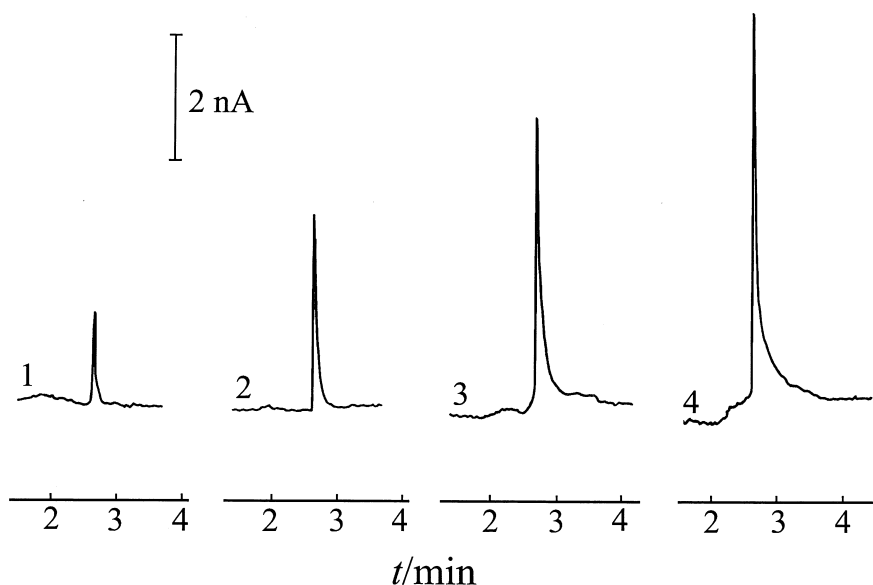
Fresh human blood was collected in a 5 ml

Fig. 5. Electropherograms of chloramphenicol in the sample of human serum. The concentration of chloramphenicol (mol/l): 1: sample; 2: (1)+1.00×10^{-5}; 3: (1)+2.00×10^{-5}; 4: (1)+3.00×10^{-5}. Conditions as in Fig. 2.

centrifuge tube and centrifuged at 1000 rpm for 5 min to obtain the human serum (supernatant liquid). No peak appears on the electropherogram of the serum blank. A synthetic human serum sample containing 1.00×10^{-5} mol/l chloramphenicol was used to verify the possibility of the standard addition method. One hundred microliters of 8.4×10^{-3} mol/l HOAc−3.2×10^{-2} mol/l NaOAc was added to 10 μl serum sample. After the sample solution was diluted to 1 ml, it was injected into the CZE–electrochemical system by electromigration injection with 5 kV for 5 s. The electropherograms of the human serum sample without and with addition of the standard solution of chloramphenicol are shown in Fig. 5. The concentration of chloramphenicol in the human serum sample obtained by the standard addition method was 9.65×10^{-6} mol/l, which agrees with the value in the human serum sample. The recovery was 99%.

Acknowledgements

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